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Genomic characterization of two *Staphylococcus epidermidis* bacteriophages with anti-biofilm potential

Diana Gutiérrez, Beatriz Martínez, Ana Rodríguez and Pilar García*

Abstract

Background: *Staphylococcus epidermidis* is a commensal bacterium but can colonize the hospital environment due to its ability to form biofilms favouring adhesion to host tissues, medical devices and increasing resistance to antibiotics. In this context, the use of phages to destroy biofilms is an interesting alternative.

Results: The complete genomes of two *Staphylococcus epidermidis* bacteriophages, vB_SepiS-philPLA5 and vB_SepiS-philPLA7, have been analyzed. Their genomes are 43,581 bp and 42,123 bp, and contain 67 and 59 *orfs*. Bioinformatic analyses enabled the assignment of putative functions to 36 and 29 gene products, respectively, including DNA packaging and morphogenetic proteins, lysis components, and proteins necessary for DNA recombination, regulation, modification and replication. A point mutation in vB_SepiS-philPLA5 lysogeny control-associated genes explained its strictly lytic behaviour. Comparative analysis of phi-IPLA5 and phi-IPLA7 genome structure resembled those of *S. epidermidis* ϕ PH15 and ϕ CNPH82 phages. A mosaic structure of *S. epidermidis* prophage genomes was revealed by PCR analysis of three marker genes (integrase, major head protein and holin). Using these genes, high prevalence (73%) of phage DNA in a representative *S. epidermidis* strain collection consisting of 60 isolates from women with mastitis and healthy women was determined. Putative pectin lyase-like domains detected in virion-associated proteins of both phages could be involved in exopolysaccharide (EPS) depolymerization, as evidenced by both the presence of a clear halo surrounding the phage lysis zone and the phage-mediated biofilm degradation.

Conclusions: *Staphylococcus epidermidis* bacteriophages, vB_SepiS-philPLA5 and vB_SepiS-philPLA7, have a mosaic structure similar to other widespread *S. epidermidis* prophages. Virions of these phages are provided of pectin lyase-like domains, which may be regarded as promising anti-biofilm tools.

Background

Staphylococcus epidermidis is a common skin and mucous commensal of healthy humans, and can easily be transmitted to medical devices being a serious clinical problem and one of the major causes of nosocomial infections [1] as well as mastitis in lactating women [2]. In the animal health context *S. epidermidis* has also been recognized as one of the main etiological agents of ovine and bovine mastitis [3].

S. epidermidis is a key factor in the transmission of virulence factors and it is involved in balancing epithelial

microbiota. In contrast to *S. aureus*, *S. epidermidis* does not encode many virulence factors, but it can colonize the hospital environment due to its ability to form biofilms favouring adhesion to host tissues, medical devices and increasing resistance to antibiotics [4]. In addition, the enormous flexibility of this bacterium continuously generates continuously novel phenotypic and genotypic variants. Hospital isolates are often characterised by the carriage of several staphylococcal chromosome cassettes (SCCmec), conferring methicillin resistance [5]. Moreover, nosocomial *S. epidermidis* strains typically harbour multiple copies of the insertion sequence element IS256 in their genomes, which contribute to genetic adaptation during infection [6]. Recently, the first *S. epidermidis* pathogenicity

* Correspondence: pgarcia@ipla.csic.es

DairySafe Group, Department of Technology and Biotechnology of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Paseo Río Linares, s/n, 33300, Villaviciosa, Asturias, Spain

island (SePI), which encodes the staphylococcal enterotoxin SEC3 and SEIL, has been described [7].

The widespread use of antibiotics in both humans and animals has led to the emergence of infectious bacteria resistant to a wide range of antimicrobials that greatly hinders their treatment. As a result of the search for complementary agents to antibiotics, phage therapy has resurfaced as means to prevent and treat infectious diseases. Phages have already been tested as anti-infectives in humans and animals [8], and phage-encoded lytic proteins may also be used to inhibit pathogenic bacteria [9]. In addition, the use of phages to destroy biofilms has gained much interest over the past years [10]. However, scarce information exists regarding the role of phages in eliminating *S. epidermidis* biofilms [11,12]. This is probably due to the limited number of phages infecting this species that have been characterized so far [13-15].

We have previously isolated and characterized three phages infecting *S. epidermidis* strains which belong to the *Siphoviridae* family (vB_SepiS-phiIPLA5, vB_SepiS-phiIPLA6, and vB_SepiS-phiIPLA7) [15]. Phage vB_SepiS-phiIPLA5 (hereafter phi-IPLA5) behaved as a virulent phage, probably derived from vB_SepiS-phiIPLA6, while vB_SepiS-phiIPLA7 (phi-IPLA7) was temperate. Both phages exhibited plaques surrounded by an increasing halo zone indicative of a polysaccharide depolymerase activity [16]. Moreover, in challenge assays phi-IPLA5 had lytic capability against *S. epidermidis* [15].

In the present work, the complete genome of phages phi-IPLA5 and phi-IPLA7 has been sequenced, annotated and compared with those previously described for staphylococcal phages. Genes encoding putative depolymerase activities were identified in these genomes. In addition, a representative *S. epidermidis* strain collection has been analyzed by using a multiplex PCR and the frequency of certain prophage groups determined. This study thus provides the basis for the evaluation of phages to control *S. epidermidis* strains.

Results and discussion

Due to the renewed interest in phage therapy and the ability of phages to successfully combat infections in both animals and humans, the aim of this work was the genetic characterization of two new *S. epidermidis* phages (phi-IPLA5 and phi-IPLA7) to investigate their potential as antimicrobials and, more specifically, as anti-biofilm agents based on our previous observations of the presence of an increasing halo surrounding the lysis plaques, indicating a depolymerase activity [15].

Genome overview of phi-IPLA5 and phi-IPLA7 phages

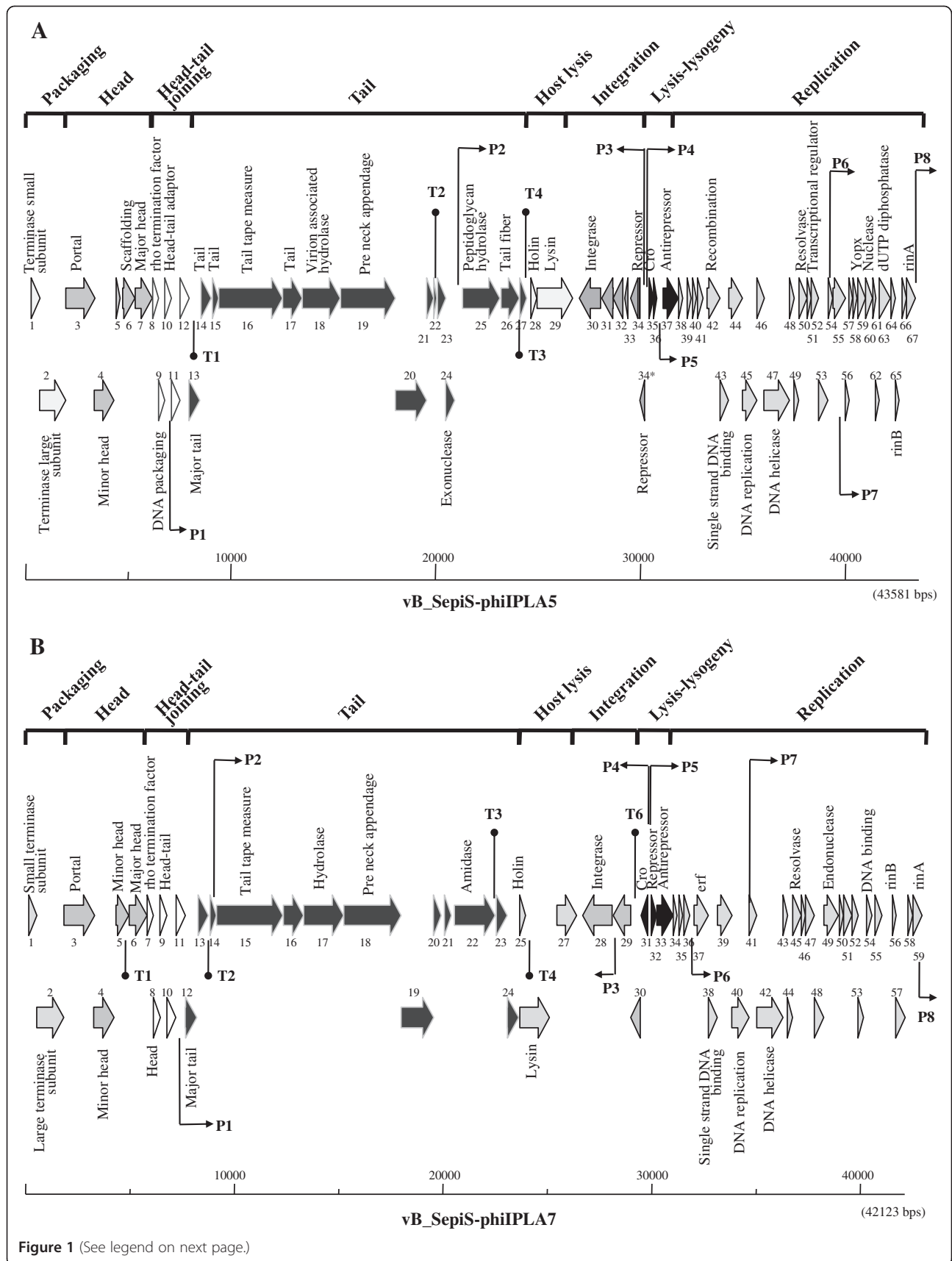
Both phages have a linear, double-stranded DNA genome consisting of 43,581 bp encoding 67 putative *orfs* in phi-IPLA5, while the phi-IPLA7 genome was 42,123 bp

and 59 putative *orfs* were identified (Additional file 1: Table S1 and Additional file 2: Table S2; Figure 1A and B). The G+C content of phi-IPLA5 and phi-IPLA7 was 34.7%, which is slightly higher than that of *S. epidermidis* strains (32%) [17]. A BLASTN search revealed that nucleotide sequence of phi-IPLA5 and phi-IPLA7 shared a high degree of similarity with the other two *S. epidermidis* phages phiPH15 and phiCNP82 (64% and 65%, for phi-IPLA5, and 81% and 67% for phi-IPLA7, respectively) [14]. Bioinformatic analysis revealed a similar organization of the two phages in five functional modules (packaging, structure/morphogenesis, host lysis, lysogeny and replication/regulation) that perfectly fits the general structure of most double-stranded DNA bacteriophages [18]. Several putative promoters and terminators in phi-IPLA5 and in phi-IPLA7 were found by searching for the *S. aureus* σ^{70} -dependent promoter consensus motif (Figure 1A and B, Additional file 3: Table S3). The deduced promoter positions are consistent with a modular arrangement and imply that there is modular control of gene expression. The putative transcription pattern of both phages reveals two main groups of *orfs* running divergently and one of them includes the lysogenic cassette.

The amino acid (aa) sequences of the predicted *orfs* were searched for similarities to sequences from the available databases (Additional file 1: Table S1 and Additional file 2: Table S2). Significant matches were obtained for 36 *orfs* from phi-IPLA5 and 29 *orfs* from phi-IPLA7 and biological functions were assigned. No tRNA genes were found. No virulence genes were clearly identified.

In the DNA packaging module, the putative large terminase subunit of phi-IPLA5 and phi-IPLA7 showed homology (59%) with those belonging to the *pac*-type phages such as *Bacillus subtilis* phage SPP1. It has been suggested that different functional classes of phage-encoded terminases can be predicted from their amino acid sequence [19]. Confirmation of this result was obtained by restriction analysis of phi-IPLA5 and phi-IPLA7 DNAs with the endonuclease *Xba*I. The phage genomes both have two cut sites but produced two single bands on agarose gels (data not shown), which suggests that both phi-IPLA5 and phi-IPLA7 genomes were circularly permuted.

In the structural module, the predicted major head and major tail proteins had a molecular mass consisting with previous protein analysis of virion particles, which showed a major polypeptide (34 kDa) in phage phi-IPLA5 and two main proteins (27.5 and 34 kDa) in phi-IPLA7, respectively [15]. Putative virion-associate hydrolases (phi-IPLA5 gp18 and phi-IPLA7 gp17) with an aminoterminal endopeptidase tail domain and a SGNH hydrolase domain related with lipases and esterases, were identified. Finally, pre neck appendage proteins (phi-IPLA5 gp19 and phi-IPLA7 gp18) with pectin-lyase like domains were identified



(See figure on previous page.)

Figure 1 Physical and genetic map of phages phi-IPLA5 (A) and phi-IPLA7 (B). The ORFs are sequentially numbered, indicated by arrows proportional to their lengths and pointing toward their direction of transcription. Some ORFs have been placed below for clarity. The functional modules are indicated on top of the scheme, and the names of several putatively or experimentally identified genes are shown. Putative promoter (P) and terminator (T) sequences are also indicated.

that could also be involved in the extracellular material degradation.

Phage phi-IPLA5, although strictly lytic, encoded a deficient lysis-lysogeny module. The phi-IPLA5 gp34 and phi-IPLA5 gp34* proteins shared extended similarity with repressors of the CI type. A one-base replacement that shifted a TAC codon to the stop codon TAA was mapped at 29941 nt resulting in a second *orf* (*orf34**). No RBS upstream of *orf34** could be detected. The presence of a truncated CI repressor in phi-IPLA5 would explain why the phage was unable to lysogenize [15]. In the *cl-cro* intergenic regions of both phages, two adjacent and outward-facing putative promoters for *cro* and repressor genes were identified (Figure 1A and B, Additional file 3: Table S3). Additionally, two 7-bp direct repeats overlapping the two putative promoters in phi-IPLA7 were recognized (data not shown). These sequences might be putative operators for the binding of CI repressor which have been reported as regulators in the lysogeny module gene expression [20].

In the replication module, both phages contained DNA replication proteins (phi-IPLA5 gp45 and phi-IPLA7 gp40) with DnaB domains which are essential in replication initiation, as well as DnaD domains which are a component of the primosome. phi-IPLA5 gp50 and phi-IPLA7 gp45 displayed homology to a Holliday junction resolvase (RusA) [21]. phi-IPLA5 gp59 had homology with Yopx proteins, an uncharacterized, well-conserved family of proteins found in bacteriophage and prophage regions of Gram-positive bacteria. A putative dUTPase gene gp63 was predicted in phi-IPLA5 genome which is highly conserved in several staphylococcal and lactococcal phages [22,23]. Gp65 and gp67 from phi-IPLA5 and gp56 and gp59 from phi-IPLA7 displayed similarity to the RinA and RinB family of transcriptional regulators. Recently, the RinA family proteins have been showed as activators required for transcription of the late operon in temperate staphylococcal phages [24].

Comparative genomics of phi-IPLA5 and phi-IPLA7 phages

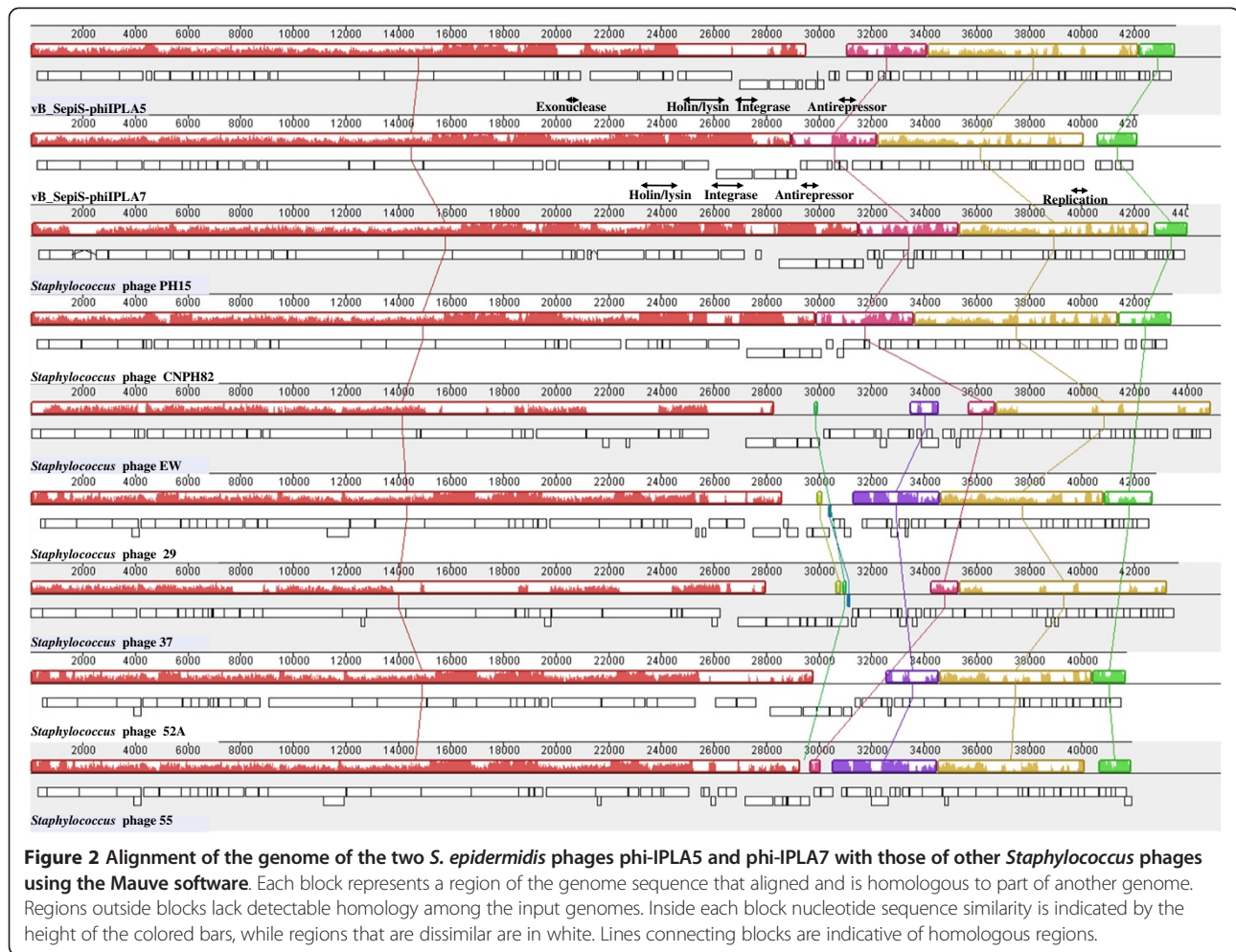
BLASTN database searches with the complete genome sequence of phi-IPLA5 and phi-IPLA7 revealed similarity at the nucleotide level with the two previously described *S. epidermidis* phages PH15 and CNPH82 and with *S. aureus* phages such as phiEW, phi29, phi37, phi52A and phi55. Comparison of these phages using Mauve software revealed gene synteny among the four *S. epidermidis* phages. Four homology blocks mostly matched the morphogenesis and

integration, the lysis-lysogeny, replication, and regulation modules, (Figure 2). Extensive similarity in the head and tail morphogenesis modules was observed among the *S. epidermidis* phages genomes and other *Staphylococcus* phages (Figure 2; Additional file 1: Table S1 and Additional file 2: Table S2). In fact, *S. epidermidis* phages PH15 and CNPH82 have been shown to be highly similar to class II clade C of *S. aureus* phages [14]. An exception was observed in the putative exonuclease encoding gene (*orf24*) from phi-IPLA5, which was not found in phi-IPLA7 or in other staphylococcal phages. The phi-IPLA5 lysis region seemed to be rather unique, specifically in the holin encoding gene (*orf28*), to which no similarity was detected within other *S. epidermidis* phages. The holin phi-IPLA5 gp28 protein showed similarity (50%) to the holin from *S. aureus* phage 29 while the holin phi-IPLA7 gp25 protein was identical to those in phages PH15 and CNPH82. Similarly, the phi-IPLA5 lysin encoding gene (*orf29*) did not have any matching gene in other phages. Moreover, the lysin phi-IPLA5 gp29 protein belongs to the amidase_2 family while those of phi-IPLA7 (gp26) and the lysins from *S. epidermidis* phages PH15 and CNPH82 were highly similar (99%) and belong to the amidase_3 family. A similar pattern was observed in the region surrounding the integrase gene. The putative integrase gene (*orf30*) of phi-IPLA5 differed from that in phi-IPLA7, and the corresponding protein (gp30) was 70% similar to that of *S. aureus* phi96, while the integrase phi-IPLA7 protein (gp28) resembled that of *S. epidermidis* PH15 and CNPH82. The phage integrases from the newly isolated phages belonged to different families (Additional file 1: Table S1 and Additional file 2: Table S2).

Finally, no similarity with other phages was detected for the antirepressor encoding gene (*orf33*) found in the phi-IPLA7 lysis-lysogeny region. Based on these data, *S. epidermidis* PH15 and CNPH82 and *S. aureus* phages phiEW, phi29, phi37, phi52A and phi55 are the closest relatives to phi-IPLA5 and phi-IPLA7. The extensive similarity between these phages is mainly in the morphogenetic region as previously reported on other *Siphoviridae* phages [25]. The observed homology in other regions or modules supports the modular theory of phage evolution [26].

Incidence and typing of prophages in *S. epidermidis* strains

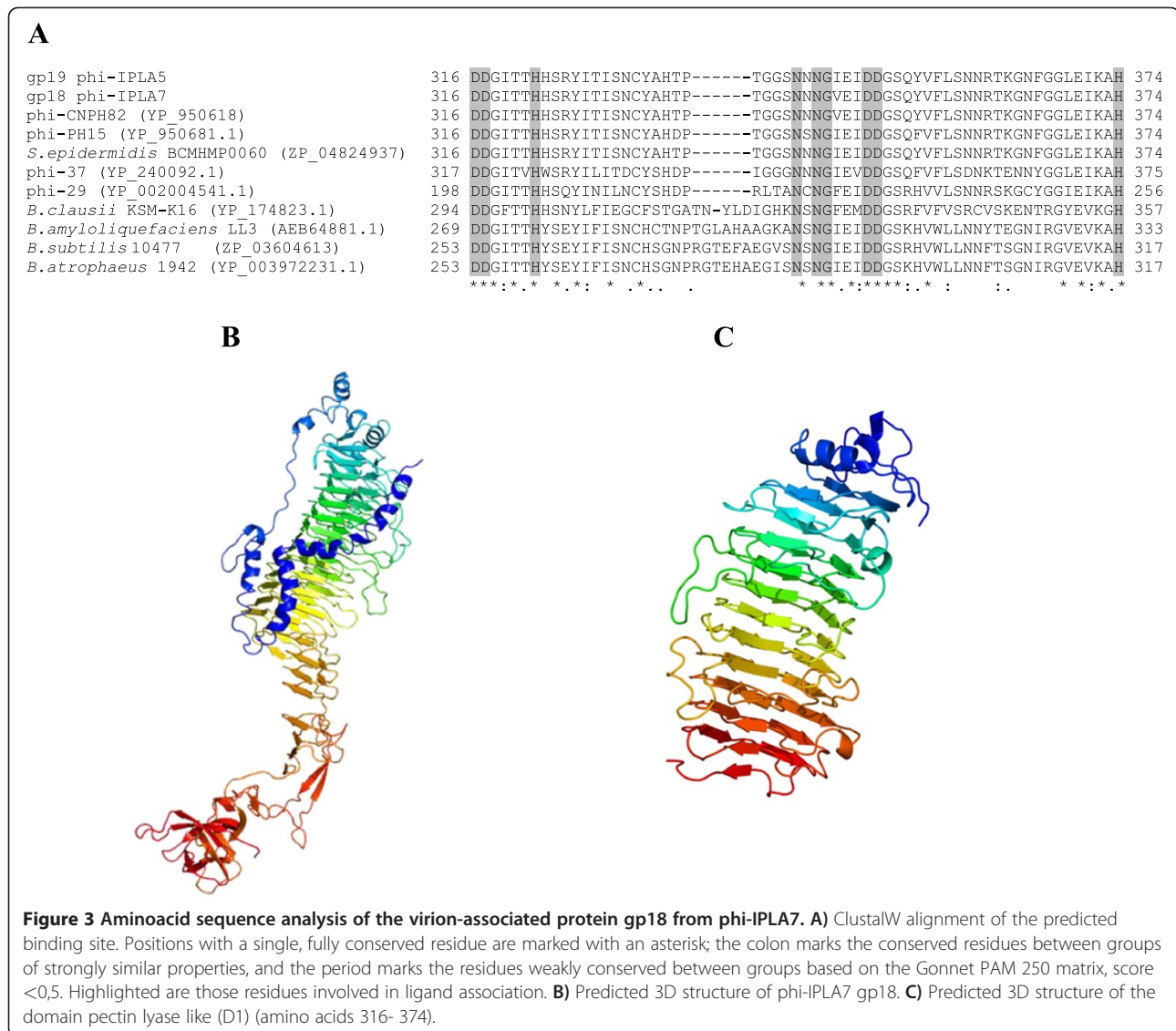
Previous studies have shown that prophages integrated in the bacterial chromosomes are the most widespread mobile genetic elements in *S. aureus* strains, which typically



carry between one and four prophages [27]. However, the prophage content in *S. epidermidis* has not been determined to date. To approach this, the integrase (*int*), holin (*hol*) and major head protein (*mhp*) genes were selected as marker modules as previously described in by [28] who studied genome mosaicism in prophages of *S. aureus*. Based on the genome wide comparison among phages and further *in silico* analysis of the integrase genes from the four *S. epidermidis* phages (phi-IPLA5, phi-IPLA7, PH15 and CNPH82), two groups were identified: int1 comprised by the highly similar *int* genes from phi-IPLA7, PH15 (95%) and CNPH82 (99%) and int2 composed by *int* phi-IPLA5 to which no similarities were found among them. Likewise, *S. epidermidis* phage holin genes defined two groups: hol1 group comprised by *hol* phi-IPLA7, PH15 (91%) and CNPH82 (100%) and hol2 group that included *hol* phi-IPLA5. Finally, the phi-IPLA7 *mhp* gene was very similar to phi-IPLA5 (97%) and PH15 (95%) but no counterpart was identified in CNPH82. Based on amino acid sequence homology this protein belongs to the phage-

capsid superfamily. The presence or absence of this gene generated the mhp1 and mhp2 groups, respectively.

The presence of prophages in our *S. epidermidis* collection containing 60 isolates from women breast milk was investigated by a multiplex PCR to amplify the above mentioned genes (*int*, *hol* and *mhp*). Prophages of the group mhp1 were the most frequent (30%) but none of the other targeted markers were amplified in these strains (Additional file 4: Table S4). About 10% of the isolates were included in the mhp2 group and were mostly associated to the int2 marker. We also observed that some phage groups were completely absent and others were less frequent. There were also some strains in which no amplification was observed pointing to the absence of prophages or the presence of other genes not detected by our PCR approach. It is remarkable that, besides the 10 *S. aureus* bacteriophage integrase gene classes analysed previously [28], many other types of modules containing lysogenic functions, DNA replication, packaging, tail appendices and host lysis were



described in *S. aureus* phages, revealing the high diversity and the mosaic structure of prophages in this species [22].

Previous studies suggested that the integrase type group in *S. aureus* strains is closely linked to the virulence gene content of prophages and might therefore convey information about their pathogenic potential [28]. We found that the largest group of *S. epidermidis* prophages belongs to int2 group, which was mostly found in mastitic strains. On the other hand, prevalence of prophages, as defined by the positive amplification of at least one marker gene, was higher (82% vs 59%) in strains producing mastitis. This result would support the hypothesis that prophages are directly involved in virulence [29]. However, no virulence factors have been described to date in *S. epidermidis* phages.

In our previous work, the yield of mitomycin C inducible prophages in *S. epidermidis* was rather low (3%) and

we hypothesized that it could be due to the lack of appropriately sensitive host strains to detect them [15]. In view of the new PCR results, phage DNA sequences were present in 73% of the analyzed *S. epidermidis* strains. Although amplification of at least one of the markers does not necessarily mean that the bacterial strains are lysogens (i.e. marker genes in defective prophages), our results support the notion that lysogeny in *S. epidermidis* may be higher than anticipated and likely more frequent among clinical strains, as noted previously in *S. aureus* strains isolated from diverse clinical samples [28].

Virion proteins with a putative hydrolytic activity of extracellular components

A pectin lyase-like domain (aminoacids 117 to 539) was identified in the pre-neck appendage protein of both phages (Additional file 1: Table S1 and Additional file 2: Table S2).

Recently, it has been demonstrated that some tail spike proteins similar to pectin lyases have exopolysaccharide (EPS) degrading activity [30]. Similar proteins to phi-IPLA5 gp19 and phi-IPLA7 gp18 were identified by BLASTp search in PH15 and CNPH82 phages, *S. aureus* phage 37, *Bacillus* phage phi29 and prophages in several *Bacillus* species (Figure 3A). Surprisingly, most of the phi-IPLA5 gp19 and phi-IPLA7 gp18 related proteins belonged to *Bacillus* sp and not to other staphylococcal species. It is remarkable that all the proteins analyzed were highly conserved at the predicted binding site (amino acids 316-374) and the residues responsible for the interaction with the ligand were shared by the different phages (Figure 3A). To support the predicted catalytic domains, the software Phyre² was used to predict the tridimensional structure (3day) of the protein phi-IPLA7 gp18 to which, the primary structure of the counterpart protein from phi-IPLA5 was 98% identical. To model phi-IPLA7 gp18 (Figure 3B), the crystal structure of the bacteriophage phi29 gp12 neck protein from *Bacillus subtilis* [31] was used. 82% of phi-IPLA7 gp18 amino acids were predicted at a confidence level of 90%. The pectin lyase-like domain (D1) (Figure 3C) displayed a right-handed continuous twelve helix repetition, where each coil of the helix featured three beta-strands and three turn regions. Proteins containing these repeats most often are enzymes with polysaccharide substrates, and it was demonstrated that this topology is shared by several proteins, with the variation in the number of coils, including bacterial pectate lyases, fungal and bacterial galacturonases and phage tail spikes [32]. On the other hand, no amino

acid sequence homology was detected between phi-IPLA7 gp18 and *Pseudomonas putida* φ15 tail spike protein, although this also showed right handed beta helical folds identified in carbohydrate depolymerizing enzymes [30].

Biofilm degradation by phi-IPLA5 and phi-IPLA7 phages

Three lines of evidence were pointing to a putative anti-biofilm activity by the newly isolated *S. epidermidis* phages phi-IPLA5 and phi-IPLA7. First, as previously reported [15], a halo surrounding the clear lysis plaques could be observed which increased with time. These halos are regarded as an indicator for the presence of phage-associated EPS depolymerases [16,30]. Secondly, a clear zone was previously observed when phages phi-IPLA5 and phi-IPLA7 were dropped on some bacterial strains (drop-sensitive strains). These strains were resistant to infection meaning that no plaques were observed when the phage stock was diluted and plated on these strains [15]. Third, based on sequence comparison and 3D predictions, two candidate proteins were identified as putative EPS degrading enzymes. To investigate if these phages were involved in biofilm degradation, cell counts and phage titre of the clear zone where the phage suspension is dropped, and into the host cell lawn were carried out. As expected, lower bacterial counts were found when the phages were dropped onto the sensitive strain *S. epidermidis* F12 while viable counts of the resistant *S. epidermidis* CJBP3 remained unaltered (Figure 4). Interestingly, the clear zone generated on the drop-sensitive strains revealed similar bacterial counts to the bacterial

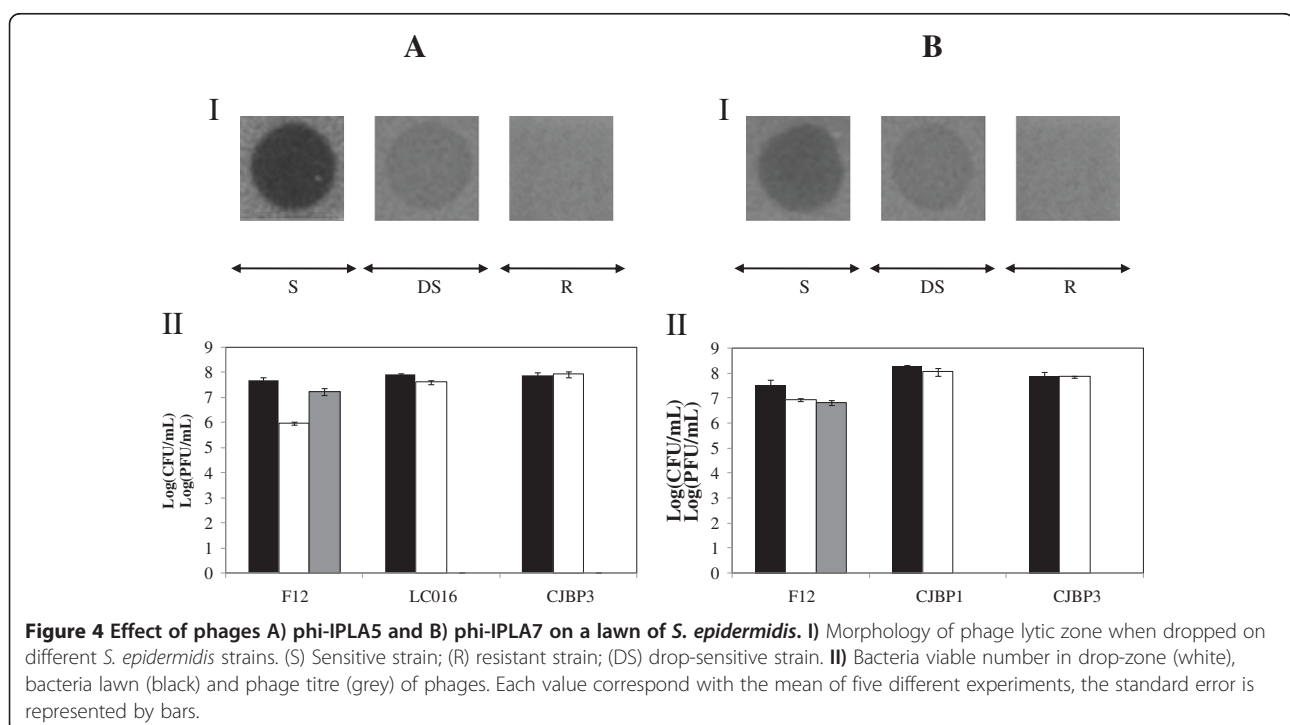


Figure 4 Effect of phages A) phi-IPLA5 and B) phi-IPLA7 on a lawn of *S. epidermidis*. I) Morphology of phage lytic zone when dropped on different *S. epidermidis* strains. (S) Sensitive strain; (R) resistant strain; (DS) drop-sensitive strain. II) Bacteria viable number in drop-zone (white), bacteria lawn (black) and phage titre (grey) of phages. Each value correspond with the mean of five different experiments, the standard error is represented by bars.

lawn despite its distinct visual appearance. Moreover, *S. epidermidis* F12 clearly supported phage propagation, while no phages were detected when sampling the drop zone of the resistant and drop sensitive strains (Figure 4). Overall, these results supports the presence of phage depolymerase activity, although we can not totally discard our previous hypothesis that ascribed the clear zone on drop-sensitive strains to “lysis from without” [15].

In order to confirm the ability of the phages to mediate biofilm degradation, the three types of strains were grown in microtiter plates for 7 days for biofilm formation and subsequently challenged with phage phi-IPLA7. Viability of the sensitive *S. epidermidis* F12 in both planktonic and biofilm cells was readily reduced even after 1 h in the presence of the phage, and only 5% of the total counts survived (ANOVA; $p < 0.05$) after 3 h (Figure 5). In the case of the drop-sensitive *S. epidermidis* CJBP1, total cell numbers remained unchanged (ANOVA; $p > 0.05$) at both 1 h and 3 h. However, a dramatic shift towards the planktonic state was observed in the presence of the phage and, after 3 h, and just 2% of the cells remained attached (Figure 5B). These data support the ability of phage phi-IPLA7 to degrade the extracellular material of the biofilm, releasing the attached cells. On the contrary, neither the viability nor the biofilm formed by the resistant strain *S. epidermidis* CJBP3 was affected by phage phi-IPLA7 (ANOVA; $p > 0.05$). A different composition in the extracellular biofilm material could explain this result. Studies on staphylococcal biofilm development suggest that the extracellular matrix consists of proteins, DNA, and/or polysaccharide (PIA) [1]. Recently, it has become evident that some strains are not reliant on PIA for biofilm formation [33].

Conclusions

In this work, we have presented a detailed genomic and molecular characterization of two new *S. epidermidis* phages, phi-IPLA5 and phi-IPLA7. Based on this, a

multiplex PCR was designed that revealed a high prevalence of prophage and/or defective prophages within *S. epidermidis* strains. Genome mining detected the presence of virion-associated proteins with a putative EPS depolymerase activity. To our knowledge, this is the first time that degradation of extracellular material has been ascribed to *S. epidermidis* phages. Further studies to confirm the role of these proteins in phage-induced biofilm destructuring are in progress with the hope to set the foundation of new anti-biofilm strategies.

Methods

Bacterial growth conditions and phages propagation

S. epidermidis F12 was used as the host strain for phages phi-IPLA5 and phi-IPLA7 [15]. A total of sixty *S. epidermidis* strains isolated from women’s breast milk [2] were used in lysogeny analysis (Additional file 5: Figure S1). Staphylococcal cells were routinely cultured in TSB broth (Tryptona Soy Broth, Scharlau, Barcelona, Spain) at 37°C with shaking or in TSB plates containing 2% (w/v) bacteriological agar (TSA). Bacteriophages phi-IPLA5 and phi-IPLA7 were propagated as described previously [15].

Phage genome sequencing and analysis

To prepare bacterial DNA-free samples for sequence analysis, the purified phages were treated with DNaseI bovine pancreas (Sigma, Madrid, Spain) and the phage DNA was extracted as previously described [34]. The genome sequence of phages phi-IPLA5 and phi-IPLA7 was generated by ultra-high throughput GS FLX sequencing with 20-fold redundancy on average. *Orfs* were predicted with Clone Manager 7 version 7.10 software in all reading frames with a threshold of 40 codons. BLASTX and BLASTP (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) were used to search for homologous proteins. Structural predictions and motif searches were performed with InterProScan (<http://www.ebi.ac.uk/InterProScan/>). The search for putative tRNA encoding genes was

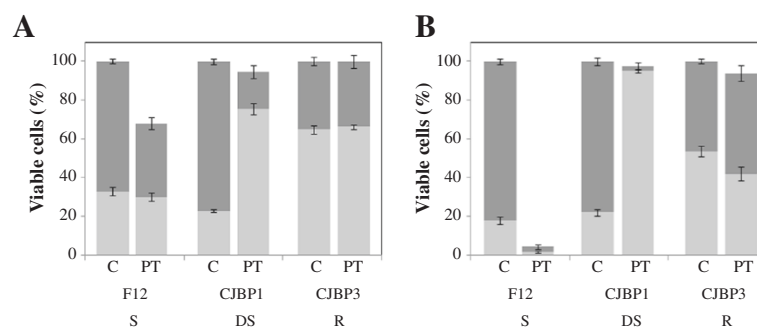


Figure 5 Killing of *S. epidermidis* cells forming a biofilm on microtiter wells by phage phi-IPLA7 after incubation for 1 h (A) and 3 h (B). Results are depicted as the percentage of attached cells (dark gray square) and planktonic cells (light gray square) detected in control biofilms treated with SM buffer (C) and in biofilms treated with phage phi-IPLA7 (PT). Each value corresponds with the mean of five different experiments and the standard error is represented by bars.

performed with tRNAscan-SE 1.21 (<http://selab.janelia.org/tRNAscan-SE/>). σ^{70} promoter sequences were identified using PPP (Prokaryotic Promoter Prediction at http://bioinformatics.biol.rug.nl/websoftware/ppp/ppp_start.php). The rho-independent terminators were identified using the Trans Term program (<http://nbc3.biologie.uni-kl.de>) and energy was calculated by the mfold web server (<http://mfold.rna.albany.edu>). Genomic comparisons at the nucleotide level were made with Mauve software, using a progressive alignment with default settings (<http://gel.ahabs.wisc.edu/mauve/>). 3 day structure prediction of proteins was made by using the bioinformatic software Phyre² (<http://gel.ahabs.wisc.edu/mauve/download.php>). To predict the binding site of the ligands, the software 3Dligand-site was used (<http://www.sbg.bio.ic.ac.uk/3dligandsite/>).

Accession numbers

The sequences of phi-IPLA5 and phi-IPLA7 have been deposited in the GenBank under accession numbers JN192400 and JN192401, respectively.

Lysogeny and prophage typing

The presence of resident prophages was screened by multiplex PCR using the primers specified in Additional file 5: Figure S1. Total DNA extraction was carried out from *S. epidermidis* strains by using "GenElute™ Bacterial Genomic DNA Kit" (Sigma-Aldrich, Madrid, Spain). PCR reactions were performed using the kit 'PureTaq Ready-To-Go™ PCR Beads' (GE Healthcare, Munich, Germany). As positive control, pure phage DNA from phi-IPLA5 and phi-IPLA7 was used. Gel images were processed using the software Quantity One software (BioRad Laboratories, Hercules, CA). The similarity matrix was calculated on the basis of the simple matching coefficient, and its corresponding dendrogram was deduced using the unweighted pair group method with arithmetic averages.

Microbiological analysis of phage lysis zones and biofilms

To visualize the lysis area formation, 5 μ l of a 10^8 PFU/ml phage suspension was dropped on a *S. epidermidis* bacterial lawn and incubated at 37°C. For comparison between the lysis zone and the bacterium lawn zone, an area with the same volume was removed from each zone, suspended in 200 μ l of SM buffer (20 mM Tris-HCl, 10 mM MgSO₄, 10 mM CaCl₂, 100 mM NaCl, pH 7.5) and vigorously vortexed. Phage titre was determined using the double layer agar method, while bacterial count was determined by plating serial dilutions on TSB agar. To determine the potential of phages to degrade biofilms, *S. epidermidis* o/n cultures were diluted in fresh TSB to 10^6 CFU/ml, poured into a 96 microwell plate (Thermo Scientific, Madrid, Spain) and incubated during 7 day at 37°C. Wells were washed twice with SM buffer and either 220 μ l of a phage stock (10^8 PFU/ml) or 220 μ l

of SM buffer were added for test and control purposes, respectively. Plates were incubated for 1 h and 3 h and then supernatants and adhered cells were collected and plated for bacteria counting. The results were represented as the viable cells percentage respect the total cell number in the control wells without phage treatment (cells in the supernatant + cells adhered to the well).

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Bonferroni multi-comparison test. Statistical significance was considered at $p < 0.05$.

Additional files

Additional file 1: Table S1. Features of bacteriophage phi-IPLA5 *orf*s, gene products (gp) and functional assignments.

Additional file 2: Table S2. Features of bacteriophage phi-IPLA7 *orf*s, gene products (gp) and functional assignments.

Additional file 3: Table S3. Putative promoters and terminators sequences of phi-IPLA5 and phi-IPLA7. -10 and -35 boxes are underlined. Nucleotide positions and presence of the TG dinucleotide were also indicated. At terminator sequences nucleotides in the stem-loop structure are underlined.

Additional file 4: Table S4. Primers used for multiplex PCR reactions.

Additional file 5: Figure S1. Multiplex PCR detecting the integrase, holin and major head protein genes in *S. epidermidis* strains. (H) Strains isolated from healthy woman, (M) strains isolated from mastitic women. Absence and presence of a specific gene is represented by white and grey boxes, respectively.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DG and PG performed bioinformatics analyses of nucleotide and protein sequences. PG, BM and AR designed the study, obtained funding and wrote the manuscript. All authors read and approved the final manuscript.

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